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REVIEW ARTICLE

Role of Protein Kinase C in Eosinophil Function

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ABSTRACT

Protein kinase C (PKC) isoforms are being elucidated as an increasingly diverse family of enzymes involved in the downstream signal transduction and cell function in various types of cells. To date, 11 PKC isoforms have been identified; they are grouped according to their molecular structure and mode of activation: conventional PKCs (α, βI, βII, and γ), novel PKCs (δ, ε, μ, θ, and η), and atypical PKCs (ζ, and ι/λ). Eosinophils are involved in the pathogenesis of allergic diseases such as bronchial asthma, pollinosis, and atopic dermatitis as well as in the inflammatory response to parasitic infections. Recent studies using selective activators and inhibitors of individual PKC isoforms have revealed that this enzyme is involved in eosinophil dynamics such as cell motility and other functions. However, the role of PKCs in eosinophil functions has been not wholly understood. In this review, we have focused upon and summarized the current knowledge regarding the role of PKC isoforms in eosinophil functions.

KEY WORDS

eosinophils, PKC inhibitors, PKC isoform, protein kinase C(PKC), signal transduction

INTRODUCTION

Protein kinase C (PKC) is a family of serine/threonine kinases that play key, distinct roles implicated in major cellular functions in a variety of cell types. The PKC activity was first defined as a histone kinase activity in the rat brain, which could be activated by limited proteolysis.[1] This kinase could also be activated by phosphatidylserine (PS) and diacylglycerol (DAG) in a Ca^{2+}-dependent manner as well as by tumor-promoting phorbol esters such as 4-phorbol 12-myristate 13-acetate (PMA).[2] It has been elucidated that PKCs play an important role in the signal transduction pathway in various cells.

Based on the similarity in the primary amino acid sequence and domain structure, at least 11 PKC isoforms have been identified in mammalian tissues to date. The distinct PKC isoforms are grouped into three subfamilies: "conventional" or "classical" PKCs (cPKCs) that include the α, βI, βII, and γ isoforms; "novel" PKCs (nPKCs) that include the δ, ε, θ, and η isoforms; and "atypical" PKCs (aPKCs) that include the ζ and ι isoforms (the mouse homologue of PKC ι has been named PKC λ).

Eosinophils play pivotal roles in allergic diseases and responses such as bronchial asthma, allergic dermatitis, and parasitic infections.[3] These cells contribute to tissue injury and inflammation by the generation of a number of toxic products in response to various proinflammatory mediators such as toxic granule proteins (e.g., major basic protein (MBP), eosinophil peroxidase (EPO), eosinophil cationic protein (ECP), and eosinophil-derived neurotoxin (EDN)), reactive oxygen species (ROS; e.g., superoxide anion), and other mediators (e.g., cysteinyl leukotrienes (CysLTs) and platelet-activating factor (PAF)).

Using selective activators and inhibitors of individual PKC isoforms, several studies have convincingly proved that this enzyme is involved in a variety of eosinophil functions such as cell adhesion,[4-7] shape change,[8,9] chemotaxis,[8] superoxide anion generation,[10-15] degranulation,[16-19] and release of other mediators.[20-23] However, the role of PKCs in eosinophil functions has not yet been fully elucidated. In this review, we summarize the role of PKC isoforms, particularly in eosinophil functions.

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Table 1 PKC isoforms and their activators and regulatory domain structures

Subgroup	Isoforms	Activators	Regulatory domain structure
Conventional PKCs	α, βI, βII, γ	PS,*1 Ca^{2+}, DAG,*2 Phorbol ester	P*4 C1a C1b C2 Catalytic domain; DAG/phorbol ester binding; Ca^{2+} binding
Novel PKCs	δ, ε, μ, η, θ	PS, DAG, Phorbol ester	C2-like P C1a C1b Catalytic domain
Atypical PKCs	ζ, ι /λ	PIP_3, *3 PS, Ceramides	P C1 Catalytic domain

*1PS, phosphatydilserine; *2DAG, diacylglycerol;
*3PIP_3, phosphatidylinositol-3,4,5-trisphosphate; *4P, pseudosubstrate

STRUCTURE AND PROPERTIES OF PKC ISOFORMS

PKC isoforms have been classified into three subgroups (*i.e.*, cPKCs, nPKCs, and aPKCs) according to their regulatory properties, which are conferred by specific domains in the proteins (shown in Table 1). PKCs are single polypeptide chains with N-terminal regulatory domains that contain an autoinhibitory pseudosubstrate domain, two membrane-targeting modules (termed C1 and C2), and a highly conserved C-terminal catalytic domain (that contains the C3 and C4 motifs required for ATP/substrate binding and catalytic activity).[24-27] The cPKC isoforms contain two membrane-targeting modules designated as C1 and C2. The C1 domain of cPKCs contains two cysteine-rich zinc fingers termed C1a and C1b; [28] the latter is involved in the binding of DAG and PMA[29-31] The C2 domain of cPKCs binds anionic phospholipids in a Ca^{2+}-dependent manner due to the presence of several invariant Ca^{2+}-binding residues in three loops at one end of the structure.[32] Similarly, the nPKC isoforms have twin C1 domains and a C2 domain (which precedes the C1 domain in the case of nPKCs) in their N-terminal regulatory regions. However, the C2 domain-like sequence of nPKCs lacks side chains bearing Ca^{2+}-coordinating acidic residues; hence, nPKCs are maximally activated by DAG/PMA and do not require Ca^{2+}. [33,34] The aPKC isoforms lack a Ca^{2+}-sensitive C2 domain and contain only a single cysteine-rich zinc finger structure that binds neither DAG nor PMA.[33,35] As a result, neither Ca^{2+} nor DAG/PMA regulate the aPKC isoforms. On the other hand, it has been suggested that the activation of PKC ζ may depend on phosphatidylinositol 3, 4, 5-trisphosphate (PIP_3), which is mainly produced by phosphatidylinositol 3-kinase (PI3-K). PKC ζ is phosphorylated and activated by 3'-PI-dependent protein kinase 1, which binds with high affinity to PIP_3.[36]

To summarize, the cPKC isoforms (PKCs α, βI, βII, and γ) can be activated by Ca^{2+} and/or by DAG and phorbol esters. The nPKC isoforms (PKCs δ, ε, θ, and η) are also activated by DAG and phorbol esters but are independent of Ca^{2+}. Finally, the aPKC isoforms (PKCs ζ and ι) are responsive to neither Ca^{2+} nor DAG/phorbol esters, while PIP_3 has been shown to activate PKC ζ.

ROLE OF PKC ISOFORMS IN A VARIETY OF CELL FUNCTIONS

Although most of the PKC isoforms are ubiquitous in mammalian tissues, some isoforms have a disproportionate distribution in specific cells, for example, PKC α in T cells,[37] PKC β in B cells,[37] PKC γ in the brain (most abundant in the cerebellum, hippocampus, and cerebral cortex),[38] PKC η in epithelial cells,[39] and PKC θ in T cells and muscle cells.[40-42] PKCs are particularly important signaling mediators in immune cells.[43] For example, PKC β participates in the control of humoral immune responses and cellular responses of B cells.[44] PKC ε is involved in the lipopolysaccharide-mediated signaling in activated macrophages.[45] Further, another report has shown that the respiratory burst activity in neutrophils was reduced by the inhibition of p47-kD protein (a component of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase—the enzyme responsible for the respiratory burst of polymorphonuclear leukocytes (PMNs)) phosphorylation by 1-O-hexadecyl-2-Q-methylglycerol (cPKC and nPKC inhibitor). As a re-

Table 2 Effects of PKC inhibitors or activators on eosinophil function

Function	Stimulant	Effect of reagents	related PKC isoforms	Spesies	References
Adhesion	PAF *1	↑ (Bis I *2)	α, βI, βII, γ, δ, ε	Human	63
	PMA *3	↓ (Rottlerin)	δ	Human	5
	IL-5 *4	↓ (Rottlerin)	δ	Human	7
	PAF, C5a *5	↓ (myristoylated PKC ζ inhibitor)	ζ	Human	64
	Histamine	↓ (Go6976)	cPKCs	Equine	6
Shape change	IL-5	↓ (GF109203, Staurosporine)	broad	Human	9
Chemotaxis	PAF, IL-5	→ (GF109203)	broad	Human	66
Superoxide generation	PAF	↓ (Bis I, myr-ψPKC)	α, βI, βII, γ, δ, ε	Human	63
	PAF	↓ (myristoylated PKC ζ inhibitor)	ζ	Human	64
	Histamine	↓ (Rottlerin)	δ	Equine	6
Degranulation	SOS *6	↓ (Staurosporine, Calphostin C)	broad	Human	19
	C5a	↑ (Rottlerin)	δ	Human	74
	PAF, C5a	↓ (myristoylated PKC ζ inhibitor, Bis I)	α, βI, βII, γ, δ, ε, ζ	Human	64
	PAF	↓ (PMA)	cPKCs, aPKCs	Guinea pig	17
Mediator release	A23187	↓ (Staurosporine, Bis I)	broad	HL-60 cells	21
(ex. CysLTs, *7 TXB_2 *8)	PAF	↑ (Bis I)	α, βI, βII, γ, δ, ε	Human	23

*1PAF, platelet-activating factor; *2Bis I, bisindolylamleimide I; *3PMA, 4-phorbol 12-myristrate 13-acetate;
*4IL-5, interleukin-5; *5C5a, complement component 5a; *6SOS, serum-oponized sephadex beads
*7CysLTs, cysteinyl leukotrienes; *8TXB_2, thromboxane B_2

sult, PKCs appear to play a major role in the regulation of NADPH oxidase. [46]

STUDIES ON THE ROLE OF PKC ISOFORMS USING GENETICALLY ENGINEERED MICE

Recent studies on PKC isoform-selective knockout mice have revealed important insights into the function of individual PKCs in mammals; these are described briefly in the following text. (1) PKC γ, one of the most prominent PKC isoforms in the brain, has been shown to be important for the brain functions involved in learning and memory.[47] (2) Lack of PKC β leads to immunodeficiency due to impaired humoral and B cell responses.[44] PKC β also appears to be critically involved in B cell receptor-mediated survival signaling for NF-κB activation.[48] (3) PKC ε has been shown to be involved in the regulation of $GABA_A$ receptor function[49] and lipopolysaccharide-mediated signaling.[50] (4) PKC θ appears to be involved in a unique signaling pathway linking T cell antigen receptor signaling to NF-κB activation in mature T cells.[51] (5) PKC δ-deficient smooth muscle cells were shown to be resistant to the apoptosis induced by angiotensin II and endothelin-1.[52] In addition, the loss of PKC δ leads to increased antigen-induced mast cell degranulation[53] and to the prevention of B cell tolerance owing to maturation and differentiation of B cells.[54] (6) PKC ζ is important for the regulation of NF-κB transcriptional activity. Consequently, a lack of PKC ζ leads to impaired B cell receptor signaling, inhibition of cell proliferation and survival, and defects in the activation of extracellular signal-regulated kinases (ERK) and the transcription of NF-κB-dependent genes. [55,56]

In contrast to studies on knockout mice, with use of transgenic mice, in which PKCs were overexpressed, the role of individual PKC isoforms was characterized. For instance, the studies characterized PKCs α and δ as regulators of glucose transport; [57] PKCs α and θ, calcineurin-induced transactivation; [58] and PKC ζ, RelA (p65, a component of NF-κB) transcriptional activity.[59] In addition to previous knowledge, these recent genetic studies suggest that PKC-related signaling pathways participate in transcriptional control, particularly that of NF-κB in various cells.

ROLE OF PKCS IN EOSINOPHIL FUNCTION

PKC isoforms are thought to be important regulators of downstream signaling cascades that control eosinophil cellular responses. Recently, interesting findings were obtained using selective activators and/or inhibitors of individual PKC isoforms that may be involved in eosinophil functions (shown in Table 2). The findings of PKCs related to eosinophil functions are summarized below.

CELLULAR ADHESION

In the early process of eosinophils activation, circulating eosinophils roll, tether, and adhere to vascular endothelial cells. The concentration gradient of chemokines induces eosinophil transmigration through endothelial gaps and accumulation at the inflammatory site. Then, the eosinophils adhere to the appropriate ligand on the subepithelial cells.[3,60] In

these processes, cellular adhesion has been shown to be one of the most critical steps for eosinophil activation.[18] We have previously demonstrated that treatment with an antibody against β2 integrin can almost completely inhibit not only eosinophil adhesion but also the degranulation induced by a lipid mediator (e.g., platelet-activating factor (PAF)), a cytokine (e.g., IL-5), or an immunogloblin (e.g., IgG).[61,62] These findings suggest that the β2 integrin-dependent cellular adhesion, particularly αMβ2 (CD11b/CD18), that is induced by the above stimuli through the membrane receptors is critical for the effector functions of eosinophils.

We have demonstrated the effects of several PKC inhibitors, including a broad-spectrum PKC inhibitor such as bisindolylamleimide I (Bis I; inhibitor of PKCs α, βI, βII, γ, δ, and ε), peptide 20–28 (inhibitor of PKCs α and β), and a specific PKC δ inhibitor (rottlerin) or PKC ζ inhibitor (myristoylated PKC ζ inhibitor), on CD11b expression on the surface of eosinophils.[63,64] The PKC ζ inhibitors did not affect the spontaneous or PAF- or C5a-induced CD11b expression in eosinophils to the extent observed with other PKC inhibitors, while only Bis I enhanced the PAF-induced CD11b expression, as previously reported.[65] This evidence indicates the possibility that PKC isoforms other than PKCs α, β, δ, and ζ are involved in the PAF- or C5a-induced CD11b expression. Furthermore, we have shown that the PKC ζ inhibitor attenuated the PAF- or C5a-induced cell adhesion.[63] In addition, Sano and colleagues have shown that rottlerin blocked the IL-5-induced β2 integrin-dependent adhesion of human eosinophils.[7] We also found that rottlerin inhibited the PAF-induced adhesion in human eosinophils (unpublished data). These results indicate that the β2 integrin-dependent adhesion is also mediated by the activation of a PKC δ or ζ pathway. Thus, interestingly, it appears that various PKCs participate in cellular adhesion, probably due to the differences in stimulants or some experimental conditions.

SHAPE CHANGE AND CHEMOTAXIS

During the process of recruitment and activation of eosinophils in the early steps of the immune response, eosinophils are stimulated on contact with chemoattractants; this results in a change in their shape, which is a prerequisite for chemotaxis.[3] Then, the eosinophils migrate by chemotaxis/chemokinesis to the inflammatory site after being attracted by the various mediators released from inflammatory cells. A previous study has reported that Bis I had no effect on the PAF-induced eosinophil chemotaxis.[66] Recently, another study has shown that inhibitors of mitogen-activated protein (MAP) kinase blocked both eotaxin- and IL-5-induced eosinophil shape changes in a dose-dependent manner, although treatment with broad-spectrum PKC inhibitors such as Bis I or staurosporine resulted in a striking inhibition of eosinophil shape change induced by IL-5 but not by eotaxin.[9] This data indicated that IL-5 and eotaxin probably regulate eosinophil shape change via a largely overlapping signaling pathway through MAP kinase. However, involvement of PKCs is limited only to the IL-5 signaling pathway in this study.[9]

SUPEROXIDE ANION GENERATION

In inflammatory conditions such as asthma, eosinophils migrate into the airways and generate highly toxic ROS, including superoxide anion and hydrogen peroxide.[65] The superoxide-generating enzyme NADPH oxidase is a multiprotein complex that comprises the membrane-bound cytochrome b_{558} (a complex of gp91 and p22) and Rap1A and four translocatable cytosolic components ($p47^{phox}$, $p67^{phox}$, $p40^{phox}$, and rac2—a small G protein).[67] In our previous study, the nonspecific PKC inhibitor Bis I was used to detect the PKC isoforms implicated in PAF-activated superoxide anion generation.[63] In this study, we found that 3 μM of Bis I produced significant inhibition of PAF-induced superoxide anion generation; however, lower concentrations of Bis I modestly enhanced the superoxide anion generation (enhancement was not significant). Another study demonstrated that three PKC inhibitors—staurosporine, Ro318220, and Go 6983—inhibited the PMA-induced superoxide anion generation in human eosinophils.[68] These data suggest that PKCs are involved in the pathway that contributes to superoxide anion generation from eosinophils. On the other hand, Bankers-Fulbright *et al.* showed that PKCs α, βI, βII, γ, δ, and ζ are constitutively expressed in human eosinophils, and a PKC δ inhibitor blocked the IL-5- or LTB_4-mediated superoxide anion generation, indicating that PKC δ regulates the IL-5- or LTB_4-evoked NADPH oxidase activity in eosinophils.[69] By using the PKC δ inhibitor rottlerin, we also showed that PKC δ is involved in the superoxide generation from PAF-stimulated eosinophils.[70] Further, we have found that PAF activates two distinct effector pathways leading to superoxide anion generation; one is a pertussis toxin (PTX)-sensitive pathway that leads to immediate and transient adhesion-independent activation, and the other is a PTX-resistant pathway that leads to late and extended adhesion-dependent activation. We have discovered that the latter pathway evokes substantial superoxide anion generation and is mediated by PI3-K.[71] In addition, previous reports have shown that PI3-K is essential for PKC δ [72] and PKC ζ activation.[73] Taken together, these observations and our present results suggest that substantial stimulus-induced superoxide anion generation is modulated by PKCs ζ and δ, which might be activated by PI3-K. In addition, our recent study has shown that a PKC ζ inhibitor suppressed the PAF- or C5a-induced superoxide anion generation in a dose-dependent manner.[64] We com-

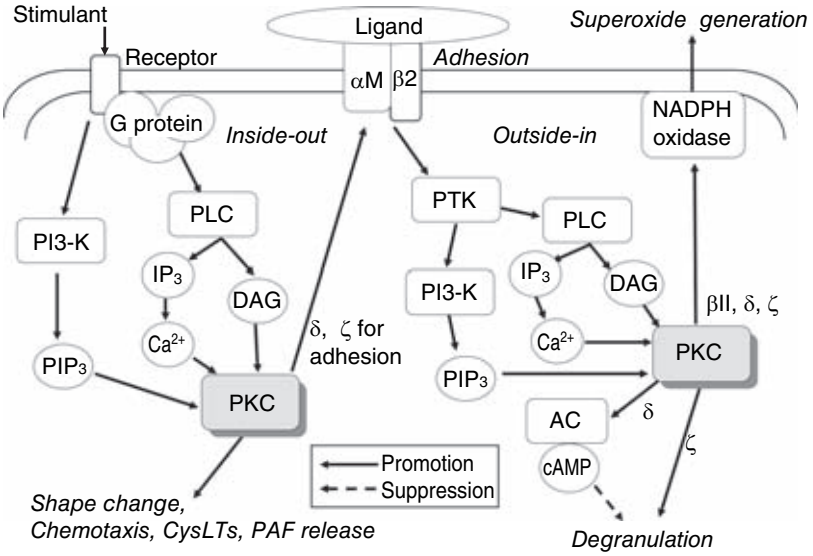

Fig. 1 The possible role of PKC isoforms in eosinophil function and its signaling pathway. Receptor-mediated stimulation by mediators induce β2 integrin expression on the eosinophil surface and a conformational change in the integrin molecule ("inside-out" signaling). Protein kinase C (PKC) isoforms other than PKCs α, β, δ, and ζ might be involved in the downregulation of the PAF-induced β2 integrin expression. Clustering and/or multimerization of αMβ2 resulting from the "inside-out" signaling is probably promoted by PKCs δ and ζ; both these isoforms are activated by phosphatidylinositol 3-kinase (PI3-K). The αMβ2-ligand interaction ignites the "outside-in" signaling, for instance, phosphorylation of tyrosine kinase (PTK) followed by activation of phospholipase C (PLC). Activated PLC catalyzes the formation of DAG, which activates cPKCs and nPKCs. Another product of PLC, inositol triphosphate (IP_3), which induces the release of Ca^{2+} from the intracellular stores, is involved in the activation of cPKCs. The αMβ2-ligand interaction evokes the activation of PI3-K, which can contribute to the activation of PKCs δ and ζ. Eventually, various eosinophil effector functions such as superoxide anion generation might be induced, at least, by PKCs β, δ, and ζ, and degranulation, by PKC ζ. Alternatively, a ligand coupled to its receptor stimulates the G protein; this is followed by the activation of second messengers and the activation of PKC isoforms such as PKC δ. This signal might cause a shape change, chemotaxis, and mediator release (cysLTs, PAF, etc) or eicosanoid production that is probably independent of cellular adhesion and outside-in signaling.

pared this effect with those produced by other PKC inhibitors such as Bis I, peptide 20–28, and rottlerin. As a result, a significant difference in the IC50 values was observed with respect to the effects of the PKC inhibitors on eosinophil superoxide anion generation; this suggests that the involvement of PKC isoforms might depend on the stimulant. Furthermore, we have demonstrated that the interception of actin assembly with cytochalasins, which are inhibitors of actin polymerization, resulted in the inhibition of eosinophil shape changes as well as the suppression of translocation of PKCs βII, δ, and ζ to beneath the cell membrane; this suggests that the cytoskeleton-related translocation of PKCs plays a critical role in the superoxide anion generation in adherent human eosinophils.[65] This evidence suggests that PKC δ or ζ and PKC βII activate the stimuli-induced substantial superoxide anion generation, and the translocation of PKCs βII, δ, and ζ might be responsible for the superoxide anion generation in eosinophils.

DEGRANULATION

Similar to superoxide anion generation, degranulation accompanied by the release of toxic granule proteins is thought to be one of the major eosinophil effector functions and may be related to the pathogenesis of allergic diseases such as bronchial asthma. The relationship between eosinophil degranulation and

PKCs was investigated in several studies.[19,64,74] In a previous study, the ECP release induced by serum-opsonized Sephadex beads (SOS) was inhibited by nonspecific PKC inhibitors such as staurosporine or calphostin C.[19] We also showed that a specific PKC ζ inhibitor attenuated the PAF- and C5a-induced EDN release from eosinophils.[64] In contrast, another report has demonstrated that the activation of PKC δ by PMA can stimulate cAMP production through adenylate cyclase (AC) and that there was a good correlation between the increase in intracellular cAMP and the inhibition of degranulation in human eosinophils.[74] This report has indicated that PKC δ activation by PMA was negatively implicated in the degranulation occurring via stimulation of cAMP production. We also found that high doses of rottlerin enhanced the PAF- or C5a-induced degranulation in human eosinophils (unpublished data).

Furthermore, we have shown that the inhibition of PKC by 3 μM of Bis I led to a reduction in the PAF-induced degranulation from human eosinophils; however, lower concentrations of Bis I enhanced the degranulation.[63] This result was similar to that obtained with PAF- or C5a-induced superoxide anion generation and degranulation.[63,64] These responses suggest that PKCs have dual modes of regulation of the PAF-evoked signaling pathway in human eosinophils.

MEDIATOR RELEASE

Eosinophils release physiologically active substances such as PAF, cysteinyl leukotrienes (CysLTs), and thromboxane B_2 (TXB_2), which are chemical mediators of inflammation. A previous report showed that staurosporine or Bis I prevented the inhibition of CysLT synthesis in an eosinophilic strain of HL-60 cells stimulated by the calcium ionophore A23187.[21] In contrast, the effect of PKC activation on CysLT synthesis and the formation of prostaglandin E2 and TXB_2 was elevated after PMA treatment; this effect was prevented by staurosporine. These results indicate that PKCs also play a role in lipid mediator release.

PARTICIPATION OF PKC ISOFORMS IN EOSINOPHIL FUNCTIONS AND ITS SIGNALING PATHWAYS

Based on the above-mentioned evidence, we formulated a hypothesis for the possible intracellular signaling mechanism and the role of PKC isoforms in the regulation of eosinophil effector functions (shown in Fig. 1). Receptor-mediated stimulation via a receptor-coupled GTP-binding protein (G protein) induce the expression of β2 integrin on the eosinophil surface and a conformational change in the integrin molecules, *i.e.*, the so-called "inside-out" signaling.[60,75] In this process, some PKC isoforms other than PKCs α, β, δ, and ζ might be involved in the downregulation of PAF-induced β2 integrin expression.[63,64] Moreover, a report recently suggested that a signaling cascade through PI3-K and PKC δ is involved in the IL-5-induced β2 integrin-dependent adhesion of human eosinophils.[76] The clustering and/or multimerization of αMβ2 is followed by its focal adhesion to appropriate ligand(s). Several PKC isoforms such as PKCs δ and ζ are thought to participate in the cellular adhesion of αMβ2 to the ligand.[7,63] Once the clustering or multimerization of αMβ2 occurs at the sites of focal adhesion, the "outside-in" signaling is triggered in the cells. Previously, we have shown that the engagement of αMβ2 induced the activation of tyrosine kinase (PTK).[62,75] It is assumed that PTK activation is followed by the phosphorylation of several proteins, including phospholipase C (PLC).[60] Activated PLC catalyzes the formation of DAG, which modulates cPKCs and nPKCs, and inositol triphosphate (IP_3) from PIP_2. IP_3 induces the release of Ca^{2+} from the intracellular stores; this released Ca^{2+} together with DAG is involved in the activation of cPKCs. On the other hand, activated PI3-K produces PIP_3, which can contribute to the activation of PKC ζ.[36] Furthermore, the PI3-K activity is essential for PKC δ activation.[72] Subsequently, the activation of PKCs might positively regulate the eosinophil functions, at least, by the involvement of PKCs βII, δ, and ζ in superoxide anion generation and of PKC ζ in degranulation. Alternatively, a ligand coupled to its receptor stimulates the G protein; this is followed by the activation of second messengers and the activation of PKC isoforms such as PKC δ. This signal might cause a shape change, chemotaxis, and mediator release (cysLTs, PAF, etc.) or eicosanoid production that is probably independent of cellular adhesion and the outside-in signaling.

In conclusion, although a great deal of evidence is being accumulated on the role of PKCs in eosinophil functions, further studies are needed to clarify the extent of their involvement using more specific inhibitors and/or activators of individual isoforms, which would be developed in the near future. The clarification of the precise role of PKCs in human eosinophils would enable the development of a pioneering strategy for the treatment of allergic diseases such as bronchial asthma.

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